



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2019; 8(4): 3122-3126  
Received: 13-05-2019  
Accepted: 15-06-2019

**Shivani L Solanki**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

**Chirag M Modi**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

**Harshad B Patel**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

**Urvesh D Patel**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

**Dixita H Bhadarka**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

**Correspondence**  
**Chirag M Modi**  
Department of Pharmacology  
and Toxicology, College of  
Veterinary Science and Animal  
Husbandry, Junagadh  
Agricultural University,  
Junagadh, Gujarat, India

## Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh, Gujarat, India

**Shivani L Solanki, Chirag M Modi, Harshad B Patel, Urvesh D Patel and Dixita H Bhadarka**

### Abstract

Six herbal plants *Aegle marmelos* L. Corraefruit pulp, *Ficus racemosa* L. bark, *Phyllanthus emblica* L. fruit pulp, *Tribulus terrestris* L. fruit, *Boerhavia diffusa* L. leaves, *Abutilon indicum* L. Sweet root have been analysed for Thin-Layer Chromatography (TLC). Qualitative phytochemical screening of all the plants was performed to explore scientific basis of ethno medicinal potential. It confirmed the presence of various phytoconstituents like alkaloid, flavonoid, saponin, tannin *etc.* Thin Layer chromatography of the hydro-alcoholic extract of all the plants was performed for the important phytochemicals like Rutin, and Gallic acid. The presence of the gallic acid and rutin was confirmed in *P. emblica* L. and *T. terrestris* L., respectively. These findings provided the evidence that polyherbal mixture of all six medicinal plants may be a potent source for some medicinally important phytochemicals and it justifies its use as a medicinal plant.

**Keywords:** Herbal plant, hydro-alcoholic extract, TLC, phytochemical screening

### Introduction

Medicinal plants have protective or curative properties due to the presence of various phytochemical compounds. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs<sup>[1, 2]</sup>. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on a use of plants and plant extracts. Plants are being an effective source of both traditional and modern medicines are genuinely useful for primary healthcare. The medicinal plants produce wide range array of bioactive molecules and rich source of medicines<sup>[3]</sup>. The chief component produced by plants are alkaloids, glycosides, flavonoids, polyphenol, saponin, steroids, tannins *etc.*<sup>[4]</sup> Since last two centuries, there have been serious investigations into the chemical and biological activities of plants and these have yielded compounds for the development of synthetic organic chemistry and the emergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents<sup>[5]</sup>.

The presence of phytochemical compounds in the plants indicates its medicinal potential. The presence of tannins shows plant possess anti-parasitic, antiviral and antibacterial activities<sup>[6]</sup>. Flavanoids are the phenolic compounds having antioxidant, anti-inflammatory, anti allergic and anticancer activities<sup>[7]</sup>. Saponins act as anti-feedant and used as adjuvant in vaccines<sup>[8]</sup>. Presence of alkaloids shows antimicrobial, anticancer, antiarrhythmic and analgesic activity<sup>[9]</sup>. Steroids acts as signalling molecules and are important against cardio tonic activity<sup>[10]</sup>. Phenols are used as antiseptic and active ingredient in some oral analgesics such as Carmex and chloraseptic spray<sup>[11]</sup>. Knowledge of the chemical constituents of plants is desirable because such information may be of great value revealing new sources of economic compounds as tannins, oils, gums, precursor for the synthesis of new chemical substances which can be used in drug<sup>[12]</sup>.

Though, herbal plant may offer a vast source of potentially useful new compounds. Thus the present investigation was aimed to investigate the pharmacognostical features and phytochemical analysis for identification and authentication of hydro alcoholic extracts of various parts of different plants *Tribulus terrestris*, *Abutilon indicum*, *Ficus racemosa*, *Boerhavia diffusa*, *Aegle marmelos* and *Phyllanthus emblica*.

## Materials and methods

### Collection and identification of herbal plants

Fruit pulp of the *Phyllanthus emblica*, root of the *Abutilon indicum*, fruit of the *Aegle marmelos*, fruit of the *Tribulus terrestris* leaves of the *Boerhavia diffusa* and stem bark of the *Ficus racemosa* were selected for the study (Table 1). The selected herbal plants were collected from the field, stored in herbarium and authenticated by a Botanist. The all specimens were present in the Herbarium, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and A.H., Junagadh Agricultural University, Junagadh, Gujarat.

**Table 1:** List of medicinal plants evaluated for thin-layer chromatography

| Sr. No. | Plants                     | Vernacular name    | Part used  |
|---------|----------------------------|--------------------|------------|
| 1       | <i>Aegle marmelos</i>      | Billi/Bael         | Fruit pulp |
| 2       | <i>Ficus racemosa</i>      | Umaro              | Bark       |
| 3       | <i>Phyllanthus emblica</i> | Amla               | Fruit pulp |
| 4       | <i>Tribulus terrestris</i> | Gokhru             | Fruit      |
| 5       | <i>Boerhavia diffusa</i>   | Punarnava / Satodi | Leaves     |
| 6       | <i>Abutilon indicum</i>    | Atibala            | Root       |

### Preparation of herbal extracts

Hydro-alcoholic extract of selected herbal plants were prepared as per the method described by Chakraborty and Ghorpade, (2010) [13]. After collection of various parts of the selected vegetative plants, they were cleaned followed by shed drying. Then powder was made using electric mixture grinder from dried pieces of parts of selected herbal plants. After soaking in methanol: water solvent (60:40) for 3 days herbal plants extract were filtered using Whatman filter paper no.1 and kept in hot air oven at 40-45°C until extract become enough dried for collection.

### Qualitative Phytochemical Analysis

All herbal plant extracts were investigated for qualitative screening of different phytoconstituents using standard test [14, 15]. Each extract of herbal plants were dissolved in different solvents like water, methanol and chloroform and then used as test solution according standard methods to detect major phytochemicals like alkaloids, flavonoids, saponins, sterol, sugars, phenols etc present in the extract.

### Detection of Alkaloids

Methanolic extract was diluted in acidic solution like 1-5% HCl or 10% acetic acid. This test solution was used for detection of alkaloid using various reagents.

#### Dragendorff's test:

1 to 2 ml of test solution was mixed with Dragendorff's reagent. Formation of bright orange precipitates confirmed presence of alkaloid in the sample.

#### Hager's test

1 to 2 ml of test solution was mixed with Hager's reagent. Formation of yellow precipitates confirmed presence of alkaloid in the sample.

#### Mayer's test

1 to 2 ml of test solution was mixed with Mayer's reagent. Formation of white or buff precipitates confirmed presence of alkaloid in the sample.

### Wagner's test

1 to 2 ml of test solution was mixed with Wagner's reagent. Formation of brown precipitates confirmed presence of alkaloid in the sample.

### Detection of Flavonoids

Extract dissolved in chloroform/methanol/n-hexane/ethyl acetate solvent can be used as test solution.

### Shinoda test

1 ml of test solution was mixed with magnesium powder and a few drops of concentrated HCl. Development of orange, pink, red to purple color with this test confirmed presence of flavonoid. By using zinc instead of magnesium, only development of deep-red to magenta color or weak pink to magenta color or no color at all indicated presence of flavonoid.

### Sulfuric acid test

1-2 ml of test solution was mixed with few drops of concentrated H<sub>2</sub>SO<sub>4</sub>. Flavones and flavonols produced a deep yellow colored solution. Chalcones and aurones produced red or red bluish solutions. Flavanones gave orange to red colors.

### Detection of Sterol

Extract dissolved in non-polar solvent like chloroform/n-hexane/petroleum ether/sometimes methanol can be used as test solution.

### Salkowski test

2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added into 1-2 ml of test solution along the side of the test tube, forming two phases and development of red color indicated the presence of sterol.

### Detection of Phenols

#### Ferric chloride test

Test solution was mixed with 1 ml of 5% (w/v) FeCl<sub>3</sub> in 90 % methanol. It was observed for blue, blue-black, or blue-green color indicating the presence of polyphenols.

### Detection of Saponins

Pinch of the each extract was dissolved in water and shaken well. Formation of foam indicated the presence of saponin in the test sample. Foam which was stable for 15 min or more then it was considered as positive.

### Detection of Sugar

Extract dissolved in polar solvent like methanol/water/ethyl acetate/n-butanol can be used as test solution.

### Molisch test

0.5 to 1 ml test solution was mixed with 1 ml of 10 % methanolic  $\alpha$ -naphthol solution and then 4 to 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub> was added along the side of the test tube. It was observed for violet ring indicating the presence of glycoside or sugar.

### TLC Analysis

Thin layer chromatography TLC of herbal plant extracts was done according to standard methods [16]. Hydro alcoholic extract (100 mg) of all herbal plants were dissolved in methanol (1ml) and centrifuged at 3000 rpm for 5 minutes. The supernatant was collected and used for HPTLC analysis. The test sample (4 $\mu$ l) and standard test were loaded as a 6-8 mm band in the 10  $\times$  10 silica gel GF254 plate using a

Hamilton syringe and CAMAG LINOMAT 5 instrument. After saturation with the solvent vapour for 30 minutes, the TLC plate loaded with test sample and the reference was kept in a TLC twin trough developing chamber and developed up to 80 mm. The developed plates were dried in air heater to evaporate the solvents from the plates. The plates were kept in a photo-documentation chamber and the images were captured in white light, UV 366nm. Then, the numbers of spotted were noted and Rf values were calculated.

#### Detection of rutin

For the detection of rutin hydro alcoholic extracts of all six plants, mixture of solvents containing ethyl acetate-formic acid-glacial acetic acid-water were taken as a mobile phase in the ratio of 100:11:11:26 (V/V). Natural product polyethylene glycol reagent (NP/PEG reagent) was sprayed on the plate and allowed for drying. 0.1% of standard rutin (SD Fine India Ltd., India) in the methanol was used as the reference standard for flavonoid analysis. The presence of rutin was confirmed by the appearance of yellow fluorescence bands at UV 254 and 366 nm.

#### Detection of gallic acid

Qualitative screening of gallic acid in all extracts was done by TLC method. Mixture of solvents containing toluene- ethyl

acetate- formic acid-methanol was used as a mobile phase in the ratio of 6:6:1.2:0.25 (V/V). After drying the plate, a mixture of natural product polyethylene glycol reagent (NP/PEG reagent) was used as spraying agent for the detection. 0.1 % of standard gallic acid (SD Fine Ltd., India) in the methanol was used as the reference standard and comparison with spot of the sample. The presence of gallic acid was confirmed by the appearance of bright purple blue fluorescent bands at UV 254 and 366 nm.

#### Results and Discussion

The qualitative analysis of phytochemicals is very essential for identifying and isolation of active compounds present in the herbal plants. In the present study, qualitative analysis was undertaken to investigate the presence of various phytoconstituents in extracts of *A. marmelos* (fruit pulp), *A. indicum* (root), *B. diffusa* (leaves), *F. racemosa* (stem bark), *P. emblica* (fruit) and *T. terrestris* (fruit) (Table 2). The results of qualitative phytochemical analysis showed presence of various phytochemicals like phenols, tannins, flavonoids, saponins, phytosterols, terpenoids and alkaloids. Similar findings were reported earlier [17, 18, 19, 20, 21, 22]. Presence of these phytochemicals may be responsible for their pharmacological activity.

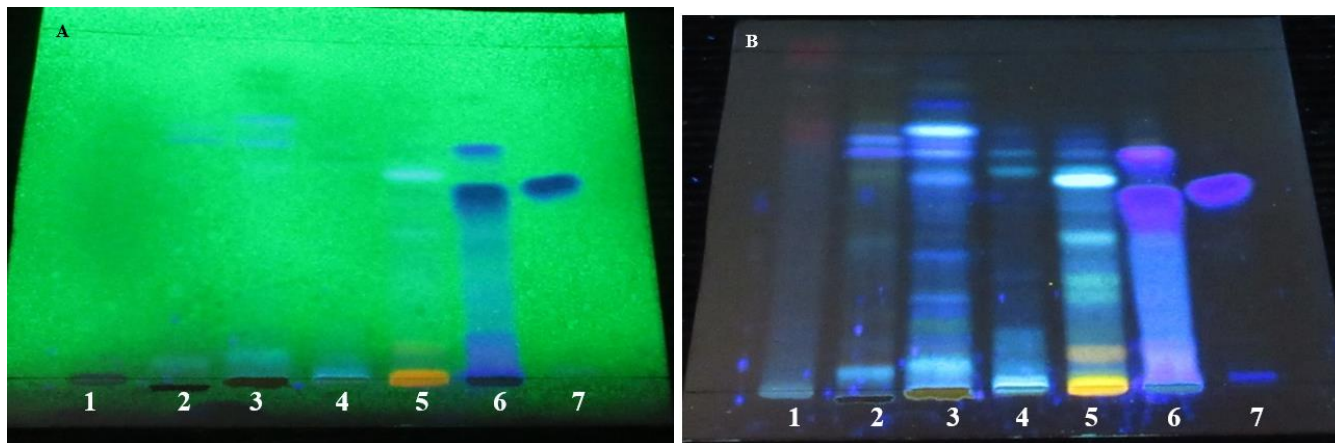
**Table 2:** Qualitative phytochemical screening of hydro-alcoholic extract of herbal plants

| Sr. No.         | Name of phytochemical/test              | 1  | 2 | 3  | 4  | 5 | 6  |
|-----------------|---|----|---|----|----|---|----|
| Alkaloids test  |   |    |   |    |    |   |    |
| 1               | (A) Mayer's test                        | -  | - | +  | ++ | - | +  |
|                 | (B) Drangendroff's test                 | +  | - | ++ | ++ | - | ++ |
|                 | (C) Wagner's test                       | ++ | + | ++ | ++ | - | ++ |
|                 | (D) Hager's test                        | +  | - | ++ | +  | - | ++ |
| Flavonoids test |   |    |   |    |    |   |    |
| 2               | (A) Shinoda test with: Mg metal         | +  | + | -  | +  | + | +  |
|                 | (B) Zn metal                            | +  | + | +  | +  | + | +  |
|                 | (C) H <sub>2</sub> SO <sub>4</sub> test | +  | + | +  | +  | + | -  |
| 3               | Saponin                                 | +  | + | ++ | ++ | + | -  |
| 4               | Sterol test                             | +  | + | +  | +  | + | -  |
| 5               | Sugars test                             | +  | + | +  | +  | + | +  |
| 6               | Phenol test                             | +  | + | +  | +  | + | +  |

**Note:** + and ++ indicate intensity of presence and – indicates absence of phytoconstituents  
Where, 1: *Aegle marmelos*, 2: *Abutilon indicum*; 3: *Boerhavia diffusa*; 4: *Ficus racemosa*;  
5: *Phyllanthus emblica* and 6: *Tribulus terrestris*

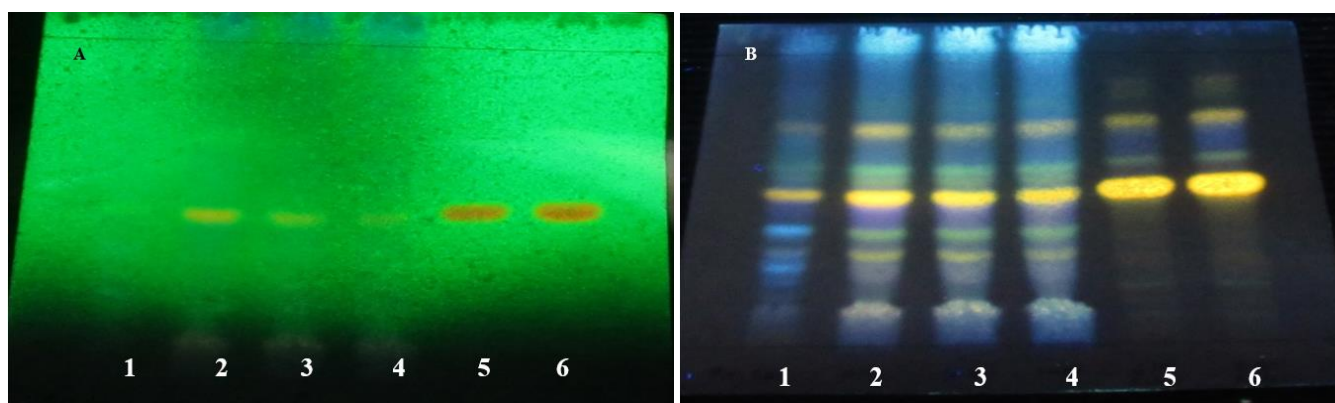
Thin layer chromatography (TLC) of all herbal plants was performed which revealed the presence of rutin in *T. terrestris* and gallic acid in *P. emblica* plant extracts at 254 nm and 366 nm. Rf values of *T. terrestris* and *P. emblica* were measured at 0.45 and 0.50, respectively which were similar to Rf values of the standard of rutin and gallic acid, respectively. Chromatogram of methanolic extract of *P. emblica* showed bright blue colour band indicated presence of gallic acid at 254 nm as depicted in Figure 1A. After spraying with NP/PEG reagent followed by drying showed bright purple

colored band of gallic acid in *P. emblica* extract (Figure 1B) at 366 nm. Thin layer chromatography of rutin from methanolic extract of *T. terrestris* showed yellow colour band at 254 nm (Figure 2A). Chromatogram of *T. terrestris* sprayed with NP/PEG reagent followed by drying showed bright yellow colour band (Figure 2B) at 366 nm. Borde *et al.* (2011) [23] and Jaijoy *et al.* (2010) [24] reported presence of gallic acid in the amla fruit by TLC.



1, *B. diffusa*; 2, *F. racemosa*; 3, *A. marmelos*; 4, *A. indicum*; 5, *T. terrestris*; 6, *P. emblica*; 7, Standard of gallic acid

**Fig 1:** Thin layer chromatogram showed presence of gallic acid in methanolic extract of *P. emblica* at 254 nm (A) and 366 nm (B) under UV chamber. Bright blue colored band indicates the presence of gallic acid in *P. emblica*.



1, *B. diffusa*; 2, *F. racemosa*; 3, *A. marmelos*; 4, *A. indicum*; 5, *T. terrestris*; 6, *P. emblica*; 7, Standard of rutin

**Fig 2:** Thin layer chromatogram showed the presence of rutin in methanolic extract of *Tribulus terrestris* at 254 nm (A) and 366 nm (B).

For the pharmacological study of newer plant based drugs, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. In the present study qualitative tests of extracts showed significant indication about the presence of metabolites. Preliminary phytochemical investigations tests are useful to isolate the pharmacologically active principles present in the plant. Plant derived natural products such as polyphenol and steroids have received considerable attention in recent years due to their diverse pharmacological properties. Different types of phytoconstituents were present in the plants like flavonoids, phenolic compounds, alkaloids which makes the plants potent to various types of ailment [25, 26].

Each extract of herbal plants were dissolved in different solvents like water, methanol and chloroform gives an impressive result that directing towards the presence of number of phytochemical. Various phytochemicals gives different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent systems.

The TLC method is best choice for the identification of secondary metabolite present in plants. The different Rf values indicate the presence of different nature of phytoconstituents in single extracts. Different Rf values of the compound also reflects an idea about their polarity. This information will be helpful in selection of appropriate solvent system for further separation of compound from these plant extracts.

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